Summary of the work

The environmental impact assessment of plastic particles may be an urgent issue considering global plastic pollution crisis. The preponderance of micro/nanoplastics in the terrestrial system might have negative impacts on plants compromising their growth and productivity. Considering the present scenario of global plastic pollution, studies of both the aquatic and terrestrial systems are important for a proper understanding of the ecotoxicological risks of micro/nanoplastics. After the release of micro/nanoplastics into the soil environment from different sources, they can interact with different components of the plant‐soil‐microbe system (such as: soil particles, natural organic matters, soil fauna, persistent pollutants and roots) and modify the soil physicochemical properties which, in turn, could affect the plant growth and development. The study of negative impacts of micro/nanoplastic on plants may represent an important aspect of their ecological risk assessment. However, the abundance of micro/nanoplastics in a soil system and their negative impacts on plants are very poorly understood due to the lack of relevant scientific literature about the terrestrial system. Therefore, our present knowledge on the phytotoxic effects of micro/nanoplastics is still in a nascent stage, which actually provokes me to work on their environmental risk assessment in onion (*Allium cepa*), a suitable model for cytogenotoxic assessment of environmental hazards.

In this work, we have used 100 nm micro/nanopolystyrenes, a dominant environmental pollutant, to investigate its cytogenotoxic effects in onion using environmentally relevant concentrations.

Polystyrene particles (100 nm), used for treatment, were mono-dispersed and spherical as observed by TEM analysis. Different tested concentrations of polystyrenes particles viz. 25, 50, 100, 200, and 400 mg/L did not exhibit any significant inhibitory effects on onion seed germination. But, the roots growth was reduced in according to increasing concentration with a remarkable root growth inhibition by ~ 50 % at 100 mg/L, indicating the morphotoxic potential of the tested polystyrenes particles. Stimulated ROS-generation in high dose groups was characterized by excess free-radical production and malondealdehide (MDA) content, as tested by biochemical analysis of superoxide and hydroxyl radicals and TBARS assay, respectively. The fortification in ROS generation and MDA content following polystyrenes treatment were dependent dose and time causing imbalance in redox-

homeostasis and oxidative-stress. Decrease in total antioxidant activity, as evaluated by DPPH assay, with increasing concentration of polystyrenes further validated the oxidative-stress due to micro-nanoplastic stress.

The fluctuation in cells number following normal mitotic event is a popularly used cytotoxic marker in cytogenotoxicity assessment of environmental toxicants. Here, I have calculated mitotic index (MI) by scoring about 5000 cells in each experimental group. A significant inhibition in MI was observed according to increasing dose and time. All tested concentrations of polystyrenes particles were recorded to have mitodepressive activity, compared to positive control, except for the treatment groups 25, 200, and 400 mg/L which showed comparable inhibitory effect on mitotic index after 24 h, 48 h, and 72 h. For genotoxicity assessment, different chromosomal and nuclear abnormalities were recorded in onion root cells comprising of meristematic and elongation zones. The prevalence of different chromosomal aberrations (CAs) was heavily dependent on time than concentration. However, the frequency of different nuclear aberrations (NAs) such as binucleate cells (BC), nuclear bud (NB) and micronucleus (MN) were dose and time dependent in all treatment groups. A remarkably high frequency of different chromosomal abnormalities including sticky bridge, c – mitosis, laggard chromosome, chromosome clumping etc. was observed in high-dose groups. All treatment groups showed a time dependent increase in the nuclear abnormality index (NAI), except for 200 mg/L, where, after 72 h of exposure, a low NAI value was noticed than that of 48 h in same treatment group. The occurrence of micronuclei by polystyrene treatment also suggests its mutagenic potential, which may be assigned to their clastogenic effect.

Inhibition of normal cell-cycle progression leads to the mitotic index reduction by blocking cells in G2 phase (Kumari et al., 2011; Sharma and Vig, 2012; Rank et al., 2002). In this regard, the DNA damage and corresponding repair pathway are important in regulating cell-cycle arrest and the faithfully transmission of an exact copy of genome to the progeny cells. Taking this into consideration, I have assessed the DNA damage potential of polystyrene particles for explaining its mitodepressive activity and the mutagenic potential, as suggested for the tested polystyrene particles. In this case, we used only 3 concentrations such as 25, 50, and 100 mg/L (named as: 25 nps, 50 nps and 100 nps, respectively) for an exposure period of 3 days; because 100 mg/L polystyrene concentration was reported to have ~ 50 % inhibitory effect on root growth. Here also we recorded a remarkably high level of oxidative-stress in high

dose groups. The increasing level of DNA damage was in accordance with the ROS generation in different treatment groups. A significantly high level of DNA-damage was recorded in 100 mg/L dose group than the control and in line with positive control group. A significantly high frequency of binucleate cells and micro-nucleus were scored in high dose group.

Now the question is - how cells respond to the DNA damage stress, imposed by polystyrene treatment. The transcript profile of the genetic markers, regulating of cell-cycle progression and DNA damage repair propose that DNA-repair pathway is activated during microplastics-induced DNA-damage stress for successful repairing and combating the stress. The regulatory role of cyclin-CDKs on cell-cycle progression is well established and in plants CDKA, also called CDC2, is the major CDK, regulating mitotic events. In this regard, it is worth mentioning that SOG1 is an important regulator that regulates the cellular abundance of mitotic CDK in plants.

We observed a high mRNA level of antioxidant genes (SOD, CAT, GPX, and APX) in low dose groups as compared to the control and high dose groups. Dose dependent decrease in CDKA and CYCA2 mRNA suggests cell-cycle arrest consistent to previous observation of mitotic index. However, a high mRNA level of SOG1 in 25 and 50 mg/L concentrations followed by a decrease in mRNA level was consistent to the expression of repressor type MYB (rep-MYB), MYB29, again suggesting cell-cycle arrest by DNA damage. On the other hand, high SOG1 and RAD51 expressions in 25 and 50 mg/L concentrations suggest the concomitant activation of a DNA-repair pathway, because SOG1 also serves as a transcription factor for CYCB1, which together with RAD51 leads to the homologous recombination dependent DNA repair. Expression profile of different anti-oxidant enzymes and cell-cycle regulators was relatively higher in low dose-group compared to high-dose group, suggesting a batter performance of plant defence system to the generated ROS and resulting DNA damage in 25 and 50 mg/L concentrations as compared to 100 mg/L concentration. The DNA-damage and the frequency of different nuclear abnormalities including micro-nucleus were assessed as markers for the detection of the mutagenic potential of polystyrene particles. Relatively high expression of activator type MYBs (MYB1, 4 and 5) in low polystyrenes concentration may regulate the anti-oxidant enzymes expression, which in turn scavenge the spare ROS mitigating DNA-damage. Expression of anthocyanin synthase (ANS) and allinase (ALLI) was also increased in low polystyrene concentrations. ANS and ALLI are involved in different anthocyanin

and allicin synthesis, which are the major phytochemicals imparting antioxidant activity in onion. These observations were consistent to the cell viability and metabolic activity, as evaluated by histo-chemical staining of root tissue with trypan blue and TTC, respectively; and here also dose-dependent response was recorded with a prominent loss of cellular viability and metabolic activity in high dose group. Together the results of mitotic index and cell-death assay confirmed the cytotoxicity of polystyrenes particles.

The present study also highlighted the potential modulatory effects of nanopolystyrene on the toxicities of heavy metals such as cadmium and mercury in terms of oxidative-stress and DNA damage. A more intense toxicological effect of Cd was recorded than nanopolystyrene and Hg, when compared individually. In case of co-exposure, it was documented that nanopolystyrene acts synergistically with $CdCl_2$ to induce ROS generation and resulting damage of DNA. Conversely, no significant change was noticed in co-treatment group, treated with $HgCl_2$ and nanopolystyrene compared to their respective individual treatment groups. The analysis of comet also showed the same result to that was obtained from DNA gel analysis in consistent with ROS generation. The tail length of individual comet was increased in samples co-treated with nanopolystyrene and $CdCl_2$ compared to the individual treatment group viz. NPS and CdCl2, which were in turn having a relatively more DNA-damage than the control sample. But in case of $HgCl_2$, no synergistic effect on DNA-damage was observed. This result of DNA damage harmonized the results of total anti-oxidant activity, measured by DPPH assay and the enzymatic activities of SOD and CAT. The activity of sod was more responsive than the cat activity under given treatment conditions. This study presents insights into the differential effects of nanoplastics on the toxicities of heavy metals; that are supposed to vary with metal species or polymer types.

Another important issue is the alterations of microtubules organization. Microtubules are the most crucial structures for regulating chromosomal dynamics during cell division. Besides DNA damage, the disturbance in microtubule organization may be one of the important reasons leading to different chromosomal and nuclear abnormalities (Singh and Roy, 2017; Datta et al., 2018). So, the alterations of microtubules organization along with DNA-damage might play pivotal role in regulating the observed chromosomal and nuclear aberrations. By immune-histochemistry and microscopy, different types of abnormal microtubule structures

such as - abnormal preprophase band and abnormal phragmoplast were detected in micropolystyrene treatment groups.

The microtubular damage, that was detected in micro-polystyrene treatment group, were further intensified under high temperature stress in combined treatment group (micro-polystyrene and high temperature), whereas, salinity stress was reported in this study to have no significant effects on the micro-polystyrenes-induced alterations of microtubule organizations. Acetylation is the most crucial epigenetic modification in regulation of microtubules stability. Similar results, where the acetylated tubulin was remarkably reduced by microplastic treatment under high temperature stress, were recorded for the acetylation of microtubules by immuno-blotting.

To track the nanopolystyrene uptake by plant roots, the nanopolystyrenes were stained with nile-red for overnight and after staining nile-red stained nanopolystyrenes were washed to remove excess stain. Then, roots were kept with NR-stained nanopolystyrenes for 3 days and the bio-accumulation was tracked in epifluorescence microscope using 490 nm emission filter. The high temperature stress further provoked the nanopolystyrenes uptake by onion roots. But, salinity stress influences the nanopolystyrenes uptake insignificantly.

The polystyrene particles, tested in this work, can inhibit the root growth but, not the seed germination indicating the morphotoxic potential in high concentrations that were well short of environmentally relevant concentration. The oxidative-stress was also recorded, which might consequently lead to the observed cytotoxicity and DNA damage. The activation of repair pathway in low concentrations of micro/nanopolystyrenes can fight well to restore the genomic integrity. However, in high concentrations, we recorded a more intense DNA damage due to an inefficient operation of the repair pathway, which indicates an alarming threat to the plant system in future in the midst of uprising micro-, nanoplastics concentration. The differential response of micro/nanoplastics in regulating heavy metal toxicity requires more study to understand its toxicological behaviour in the real environment. Formation of micronucleus and the altered microtubules organization by micro/nanopolystyrenes can showcase its mutagenic potential.